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Abstract

Neuroprotective therapy for Parkinson's disease (PD) is a treatment intended to prevent or reduce neuronal degeneration. Since clinical studies to evaluate such an effect would be prolonged, the choice of agents for use as possible neuroprotective therapy is based on the results of in vitro and animal experiments. Free radicals are currently regarded as the most important factor in the progression of PD. One current possible neuroprotective therapy is reduction of levodopa dose, since levodopa is a source of free radical formation. Dopamine (DA) metabolism inhibition, and administration of the DA agonist bromocriptine that eliminates hydroxyl free radicals have neuroprotective effects experimentally. The other candidates for neuroprotective agents are still under development. However, those whose clinical use is permitted should be considered for use, since patients with long-standing PD cannot wait until the neuroprotective efficacy of these agents is confirmed by clinical study.

KEYWORDS: free radical, scavengers, antioxidants, antiexcitotoxic, neurotrophic factors

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Review

Possible Neuroprotective Therapy for Parkinson's Disease

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Neuroprotective therapy for Parkinson's disease (PD) is a treatment intended to prevent or reduce neuronal degeneration. Since clinical studies to evaluate such an effect would be prolonged, the choice of agents for use as possible neuroprotective therapy is based on the results of *in vitro* and animal experiments. Free radicals are currently regarded as the most important factor in the progression of PD. One current possible neuroprotective therapy is reduction of levodopa dose, since levodopa is a source of free radical formation. Dopamine (DA) metabolism inhibition, and administration of the DA agonist bromocriptine that eliminates hydroxyl free radicals have neuroprotective effects experimentally. The other candidates for neuroprotective agents are still under development. However, those whose clinical use is permitted should be considered for use, since patients with long-standing PD cannot wait until the neuroprotective efficacy of these agents is confirmed by clinical study.

Key words: free radical, scavengers, antioxidants, anti-excitotoxic, neurotrophic factors

Neuroprotective therapy or neuroprotection is a treatment aimed at preventing or reducing neurodegeneration in Parkinson's disease (PD). Since there is no sensitive *in vivo* method available for assessing degeneration of the dopamine (DA) neurons affected in PD, it is difficult to evaluate the effects of neuroprotective therapy on this process. Therefore, such therapy is based primarily on the results of *in vitro* and animal experiments. Prolonged clinical trials are required to assess the efficacy of such treatment. Thus, at present, evidence for neuroprotective therapy is used largely on the basis of expectation of efficacy.

Relationship between PD and Free Radicals

It is known that oxidative stress is involved in neuronal damage. In addition to the superoxide anion (O_2^-), various other species of free radicals enhance neuronal injury (1-3). Recently, the hydroxyl radical (·OH) has been shown to injure the cell membrane directly (4, 5). Free radicals, particularly ·OH, induce peroxidation of lipids in the cell membrane and a chain reaction of damage to membrane-associated enzymes and receptors. In addition, ·OH directly modifies or destroys DNA and thereby induces cell death. Moreover, free radicals also increase the concentration of excitatory amino acids (6), causing cell death via calcium (Ca^{2+}) influx (7, 8). Free radicals are currently regarded as the most important factor in the progression of PD (4, 9). The brains of PD patients show increased lipid peroxide, iron deposition in the substantia nigra (10), and marked decreases in the levels of free radical scavenging enzymes such as reduced glutathione, glutathione peroxidase and catalase (11, 12). Thus, the generation of free radicals is likely to be increased and their elimination impaired in the brains of PD patients.

DA metabolism results in the formation of free radicals (Fig. 1). DA autoxidation or metabolism by monoamine oxidase (MAO)-B generates hydrogen peroxide (H_2O_2), which subsequently yields ·OH. However, because the content of DA is markedly decreased in the brains of PD patients, large amounts of ·OH are not expected to be generated by this pathway in patients not receiving levodopa (13). But, the administration of high doses of levodopa increases the brain DA concentration, which, in turn, may increase the abundance of ·OH and cause progressive neuronal injury (13).

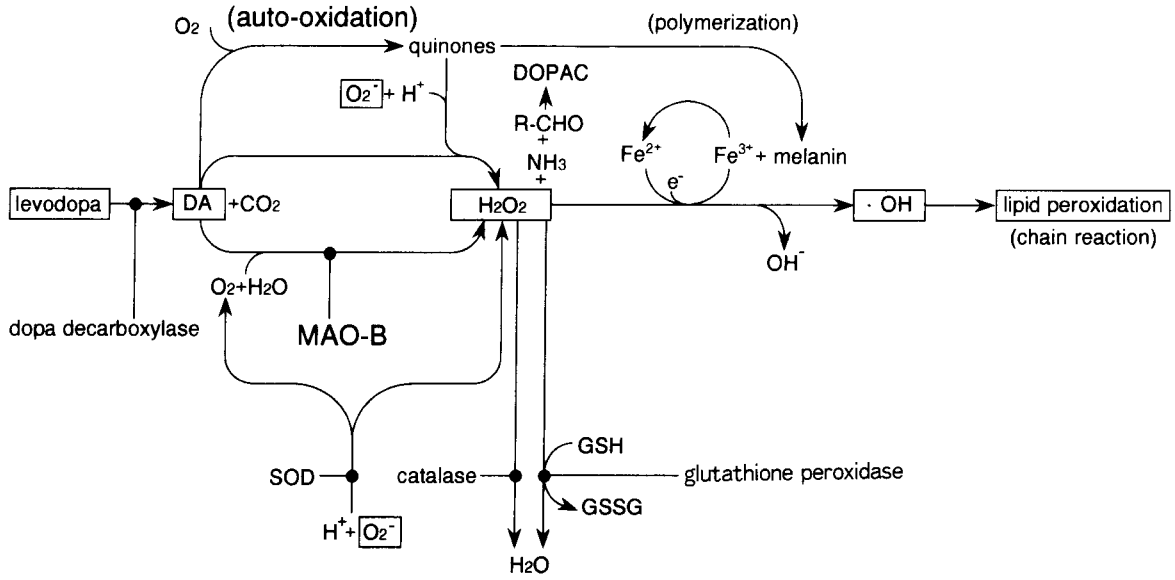

Fig. 1 Schematic representation of the possible production of hydroxyl radicals and active oxygen species from DA in the brain with consequent lipid peroxidation. DOPAC, 3, 4-dihydroxyphenylacetic acid; GSH and GSSG, reduced and oxidized forms of glutathione; MAO, monoamine oxidase.

Possible Neuroprotective Therapies

PD is a slowly progressive neurodegenerative disease with several causes, including neurotoxin exposure (14, 15) or disturbances in mitochondrial respiratory enzymes (16). As mentioned above, recent studies have suggested that free radicals may play an important role in the progression of PD (4, 9, 17, 18).

Such clinical progression could be inhibited by suppressing the formation of free radicals, protecting against oxidative stress caused by free radicals and/or inhibiting the process of neuronal death induced by free radicals. Agents antagonizing neurotoxin effect and drugs that maintain DAergic neuronal activity also may act as neuroprotective therapy. Possible neuroprotective strategies are summarized in Table 1.

Agents Related to Free Radicals

a) Restriction of levodopa and inhibition of DAergic neurons

Treatment with levodopa at high doses increases the DA levels which may, in turn, elevate free radicals (Fig. 1). Generation of free radicals may cause injury to neurons, since activity of free radical scavengers is decreased in the brains of PD patients (11, 12). Further, we have shown that levodopa is converted to a levodopa radical (19). In keeping with this hypothesis, our work has revealed that levodopa both forms a levodopa radical and inactivates endogeneous free radical scavenging activities in brain tissue *in vitro* (19). Chronic levodopa treatment of animals with a 6-hydroxydopamine (6-OHDA)-induced lesion led to increased lipid peroxide levels in the striatum (20). Although direct evidence is lacking, and the subject is controversial (21, 22), clinical and experimental studies have suggested that treatment with levodopa may accelerate the progression of PD (4, 23-28). For these reasons, the amount of levodopa administered should be minimized to decrease free radical formation. This treatment strategy is the only currently available way to reduce free radical formation in PD.

The DA agonist bromocriptine will decrease DA turnover in mice, although levodopa treatment will accelerate DAergic turnover (29). During combination therapy with levodopa and bromocriptine, the daily dosage of levodopa needed for optimal therapeutic response is significantly lower than when using levodopa alone. It is possible that the combined treatment with levodopa and a DA agonist with greater affinity for presynaptic D2 autoreceptors might maintain the normal function of

Table I Possible neuroprotective therapies for Parkinson's disease

1. Free radical-related agents
   - a) Reduce DA turnover
     - Restriction of levodopa dose
     - DA agonists
   - b) Free radical (hydroxyl radical) scavengers
     - MAO-B inhibitors
       - Selegiline (deprenyl)
       - Lazabemide
     - DA agonist (bromocriptine)
   - c) Spin traps
     - PBN
     - Salicylate
   - d) Antioxidants
     - α-Tocopherol
     - Ascorbic acid
     - EPC-K_1
     - GEPC
     - Uric acid
     - β-Carotene
   - e) Antioxidant enzymes
     - Superoxide dismutase (SOD)
     - Glutathione
     - Glutathione peroxidase
   - f) Iron chelators
2. Excitatory amino acids-related agents
   - a) Excitatory amino acid receptor blockers
   - b) Ca^{2+} antagonists
3. Neurotoxin-related agent
   - 1-Methyl-TIQ
4. Neurotrophic factors
   - Brain-derived neurotrophic factor (BDNF)
   - Glial cell line-derived neurotrophic factor (GDNF)

EPC-K_1: L-ascorbic acid 2-[3, 4-dihydro-2, 5, 7, 8-tetramethyl-2 (4, 8, 12-trimethyltridecyl)-2H-1-benzpyrene-6-yl-hydrogen phosphate] potassium salt; GEPC: L-ascorbic acid 2-(20β-11-oxo-olean-12-en-29-oic acid ethylester-3β-yl hydrogen phosphate) sodium salt; PBN: N-tert-butyl-α-phenylnitrone.

striatal DA neurotransmission for a longer period than levodopa alone. Combination therapy results in a therapeutic response equal to that achieved with high doses of levodopa, but with significantly fewer late side effects (30-32).

b) Agents inhibiting hydroxylated radicals

MAO-B inhibitors

MAO-B inhibitors (selegiline [deprenyl] and lazabemide) are thought to increase the therapeutic effects of levodopa, since MAO-B inhibitor may prevent the oxidation of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-like neurotoxin and decrease free radical formation generated by oxidation of DA. Further, MAO-B inhibitors maintain an effective concentration of DA by inhibiting DA metabolism. Animal experiments indicate that selegiline prevents development of MPTP-induced parkinsonism (33, 34) and decreases oxidative stress due to increased DA turnover (35). Although little H_2O_2 may be formed from DA in patients not receiving levodopa treatment because of abnormally low DA levels, treatment with levodopa increases DA levels which in turn may elevate the ·OH levels (Fig. 1), resulting in progressive injury to the neurons.

Selegiline has been reported to extend the life span of rats (36). In a retrospective clinical study survival is prolonged in PD patients treated with selegiline and levodopa in contrast to patients treated with levodopa alone (37).

These observation motivated the creation of a prospective, double-blind trial of combined therapy for parkinsonism using selegiline and the antioxidant α-tocopherol: "Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DETATOP) study" (38). An interim report on DETATOP study revealed that α-tocopherol had no effect, and that selegiline may have delayed the progression of PD, prolonging the period before the need to start levodopa treatment (38). However, Schulzer *et al.* (39) pointed out that findings of the DETATOP study may have reflected alleviation of symptoms rather than neuroprotection. According to the final report, symptoms did improve (40). Currently the improvement of symptoms is considered to be based on an inhibitory effect of selegiline on DA metabolism. Because the concentration of DA is maintained at a high level during the administration of an MAO-B inhibitor, O_2^- and H_2O_2 are generated by autoxidation of DA (Fig. 1). It is therefore thought to be impossible to protect against lipid peroxidation for a long period.

DA agonists. Clinical studies suggest that the DA agonist bromocriptine delays the progression of PD (41). One study has shown that the DA agonist pergolide prevents age-related decreases in DA in rats (42). Our recent work has shown that bromocriptine eliminates ·OH (29). Since ·OH is in final common pathway of the free radical formation from DA (Fig. 1), lipid peroxidation must be prevented. We found that pretreatment of mice with bromocriptine completely protected against decreases both in striatal DA and its metabolites that are induced by intraventricular injection of 6-OHDA.

Similar pretreatment with levodopa (together with carbidopa) had only a partial protective effect (29). Treatment with a DA agonist such as bromocriptine thus protects the neuronal membrane from damages both by inhibiting the formation of ·OH and by scavenging any ·OH formed (29). One can infer that levodopa, therefore should be administered to patients in conjunction with a DA agonist.

The DA agonist bromocriptine exhibits not only a therapeutic effect, but also slows the progression of disease; some patients respond to bromocriptine alone for up to 5 years (30, 43). The therapeutic effect of combined bromocriptine and levodopa therapy has been maintained for a long period (43) with no or little development of dyskinesia (30, 31). Several case reports suggest that bromocriptine not only has therapeutic effects on PD, but also blocks progression as well (30, 43).

c) Spin trapping agents

Spin trap agents, such as N-tert-butyl-α-phenylnitrone (PBN), specifically react with free radicals to form a stable radicals (44). Salicylates have been used to trap hydroxyl radicals. They have been reported to exert strong neuroprotective effects both in aging and in cerebral ischemia in animals (45), but clinical application in PD requires further examination.

d) Antioxidants

α-Tocopherol (vitamin E) is an effective antioxidant known to inhibit the chain reactions of lipid peroxidation in membranes, thereby protecting against oxidative damage (46, 47). Although α-tocopherol is hydrophobic and readily enters the brain, the brain concentration of α-tocopherol is not markedly increased by the prolonged administration of this agent in high doses (48). Also, α-tocopherol does not exhibit a neuroprotective effect in clinical practice (38).

Ascorbic acid (vitamin C) is hydrophilic and is normally present in large quantities in the plasma and the brain. Since ascorbic acid is thought to act by trapping peroxyl radicals in the aqueous phase and preventing diffusion into lipids, it is not likely that this agent, even if administered in high doses, exert neuroprotective effects in the brain.

Powerful antioxidants that combine α-tocopherol and ascorbic acid, namely L-ascorbic acid 2-[3, 4-dihydro-2, 5, 7, 8-tetramethyl-2 (4, 8, 12-trimethyltridecyl)-2H-1-benzpyrene - 6 - yl - hydrogen phosphate] potassium salt (EPC-K_1) (Fig. 2A) (49), or glycyrrhizin and ascorbic acid, namely L-ascorbic acid 2-(20β-11-oxo-olean-12-en-29-oic acid ethylester-3β-yl hydrogen phosphate) sodium salt (GEPC) (Fig. 2B) (50), have recently been synthesized. Their effectiveness is being evaluated in animal experimental models of other diseases (51). Most interesting report among them is that EPC-K_1 increases the DAergic activity (51). These new agents might have neuroprotective effect in PD.

Uric acid and β-carotene are well-characterized antioxidants that may prove effective as neuroprotective therapy.

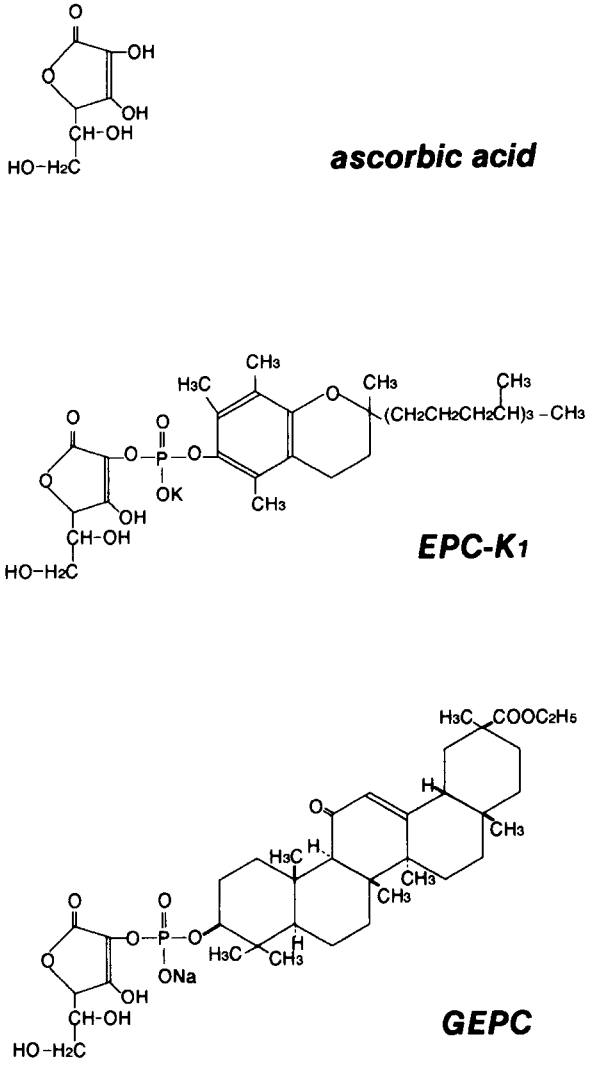

Fig. 2 Chemical structures of ascorbic acid, EPC-K_1 (L-ascorbic acid 2 - [3, 4 - dihydro - 2, 5, 7, 8 - tetramethyl - 2 (4, 8, 12-trimethyltridecyl)-2H-1-benzpyrene-6-yl-hydrogen phosphate] potassium salt) and GEPC (L-ascorbic acid 2-(20β-11-oxo-olean-12-en-29-oic acid ethylester-3β-yl hydrogen phosphate) sodium salt).

e) Antioxidant enzymes

Antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, as well as glutathione, may also be useful for scavenging oxidant species and preventing the formation of free radicals. Although SOD protects against ischemic and traumatic brain injury (52, 53), the effectiveness of SOD on PD is unclear. Increased glutathione or glutathione peroxidase activity may reduce the production of hydrogen peroxide and hence be of value in neuroprotection in PD.

f) Iron-chelating agents

Increased iron in the substantia nigra of patients with PD suggests that iron-mediated oxidative stress may contribute to neuronal death. Because iron promotes free radical formation (54), the chelation of iron may inhibit free radical generation. Thus, chelating iron may inhibit lipid peroxidation and represent a possible approach to neuroprotective therapy in PD (55–57). However, agents currently used in animal experiments, such as deferrioxamine, cannot pass the blood-brain barrier. Although iron-chelating agents that exert effects on the brain when administered peripherally have been developed, their effects after long-term use on the iron-containing enzymes tyrosine hydroxylase and monoamine oxidase in the brain and other organs have not yet been determined.

Agents Related to Excitatory Toxicity

a) Excitatory amino acid receptor blockers

The interaction of excitatory amino acids with receptors on neurons result in the opening of Ca^{2+} channels, the activation of Ca^{2+}-dependent proteases (7, 8) and nitric oxide (NO) synthase (58, 59), and, eventually cell death. Recently, glutamate receptor antagonists have been reported to provide antiparkinsonian effects in animals (60, 61). Furthermore, antagonists of N-methyl-D-aspartate (NMDA)-sensitive glutamate receptors protect against damage induced by 1-methyl-4-phenylpyridinium ion (MPP^+) and methanphetamine (62, 63). Thus, if receptors for excitatory amino acids are blocked, neuronal cell death or degenaration is inhibited when excitatory amino acids are released.

b) Ca^{2+} antagonists

Experimental studies of cerebral ischemia have demonstrated that neuronal degeneration is inhibited by Ca^{2+} antagonists (64–66). The application of Ca^{2+} antagonists to progressive neuronal degeneration in PD is also being examined.

Agents Related to Neurotoxicity

It is known that the MPP^+, formed in the brain from MPTP, or other neurotoxin such as tetrahydroisoquinoline (TIQ) (41) are thought to generate free radicals which may cause neuronal damage (17, 18). 1-Methyl-TIQ, a derivative of neurotoxin TIQ, was found to prevent DA neurotoxicity due to MPTP (67). Thus, chemical modification of neurotoxins may yield new neuroprotective agents.

Neurotrophic Factors

Some neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) have been found to specifically maintain and stimulate the DA neuron (68–70). Preclinical experiments with the use of neurotrophic factors in PD are under investigation.

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